Establishment of Yams *(Dioscorea* spp.) *In vitro* Cultures; An Initial Step of Preserving Their Genetic Diversity

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Abstract
Two experimental series were conducted. In the first experiment, explants from aerial tubers were used to introduce yams accessions into *in vitro* culture. Small aerial tubers of two yam species, obtained from 9 months old plants grown in a greenhouse were sterilized and cultivated in three different media. *D. oppositifolia* gave 68% regeneration of shoots and roots which was significantly higher than that of *D. bulbifera* (16%) 4 weeks after culture. In the second experiment, studies went on an already existing *in vitro* collection to determine the multiplication rate during storage. Thirty three yam accessions, established in two different temperature regimes, were used to determine the multiplication rate by sub-culturing single nodes with one leaf every two months. Sub-tropical/temperate yams theoretically can multiply almost as much as tropical yams. However, not all explants can further multiply.

Introduction
There exist about 600 species of yams (*Dioscorea* spp.) in the world belonging to 23 sections (Huber, 1998). Some of the species have been cultivated as food crops for centuries because of the high carbohydrate content in their tubers and some others have been used as medicinal plants due to the presence of chemical compounds (mainly diosgenin) which have been used to produce steroid drugs. The main food crop species are vegetatively propagated, therefore, preservation of yams can be suitably accomplished through *in vitro* short and medium-term conservation and cryopreservation. Successful introduction of yams into *in vitro* culture followed by multiplication would provide sufficient explants for various needs including experiments on germplasm preservation. Introducing yams into *in vitro* culture has been done using various explants such as shoot and node culture, seed culture (in case of wild species) as well as tuber culture using various culture media (Ammirato, 1982; Mitchell *et al.*, 1995a; Mitchell *et al.*, 1995b; Malaurie *et al.*, 1993; Zok *et al.*, 1998; Nair and Chandrababu, 1994). About 50 accessions of yams have been maintained *in vitro* in the *in vitro* culture and cryopreservation laboratory of the IPK genebank at Gatersleben. Information on multiplication rates of various accessions is important for their maintenance. Experiments reported in this paper are aimed at 1) introducing yams into *in vitro* culture using aerial tubers as well as assessing the effect of different culture media on the success of *in vitro* introduction and 2) determining multiplication rates of various yams accessions.

Material and methods

In vitro *introduction*
Experiment on the *in vitro* introduction was conducted during February and March 2001 using aerial tubers of two different yam accessions: Yam 16, confirmed as *D. bulbifera* L. obtained from Botanic Garden of the University of Frankfurt, Germany, and Yam 21, identified as *D. oppositifolia* L. obtained from Botanic Garden of the University of Padua, Italy. Selected small aerial tubers of Yam 16 (diameter of 1-2 cm) and the aerial tubers of Yam 21

(diameter about 1 cm) were harvested from 9 months old plants (planted in March, harvested at the end of December 2000) grown in pots in the greenhouse. Tubers were washed with water and than with 70% alcohol before being sterilized using 20% sodium hypochlorite plus 2 drops of Tween 20 on a shaker for 15 minutes. The tubers were than washed four times with sterile water before the introduction into culture. Samples of Yam 21 were mostly cultured as an entire tuber (cut into two only when necessary) while many of the tubers of Yam 16 were cut longitudinally into two (head is divided in two parts) before being cultivated in 15 ml 'introduction' medium in SIGMA glass culture tubes (length 15 cm, diameter 2.5 cm). Three different media were tested, namely I1 (MS [Murashige and Skoog, 1962] salts + 0.1 mg/l thiamine + 2 mg/l indole acetic acid [IAA] + 5 mg/l kinetin), I2 (MS salts + 0.1 mg/l thiamine + 2 mg/l IAA + 10 mg/l kinetin), and I3 (MS salts + MS vitamins + 0.2 mg/l a-naphthylacetic acid [NAA] + 0.5 mg/l 6-benzylaminopurine [BAP]). All media used 3% sucrose, 1% agar and 0.2 % activated charcoal. Each treatment combination was replicated two times by using two culture racks each consisting of 18 culture tubes. The two racks of each treatment combination were placed in different shelves in the growth chamber with 16h/8h light/dark period using fluorescent lamps, 25°C and light intensity of 60-80 $\mu mol \cdot cm^{-2} \cdot s^{-1}$. Observation was conducted during four weeks on the percentage of shoot and roots production, callus formation and the explants which showed no reaction as well as infected explants (infected explants were directly discarded from the growth chamber and were not used for calculating the percentage of each parameter). Percentage of the explants produce only roots as well as roots and callus were also noted, however, due to their very small number, they were not presented on the results. The percentage of shoot and roots production was than subjected to ANOVA to determine the main effect and the interaction between medium and genotypes (Snedecor and Cochran, 1980).

Multiplication rate

Observation on multiplication rates involved 42 clones comprising 33 accessions in 15 different species of a research collection maintained in IPK. The materials were obtained from various sources such as botanic gardens or brought by students from other countries either as *in vitro* plantlets, tubers or seeds. Most clones of the collection were introduced into culture via *in vitro* sowing as well as using nodal explants of plants grown in the greenhouse. The collection has been maintained since December 1999 by means of sub-culturing single nodal explants (with one small leaf) every two months in 15 ml of MS medium + 0.1 mg/l NAA + 2 mg/l BAP with 3% sucrose, 1% agar and 0.2% activated charcoal in 15 ml 'introduction' medium in SIGMA glass culture tubes (length 15 cm, diameter 2.5 cm). Previously, culture environment used was a 25 °C growth room with 16 h photoperiod (8 h dark) and 60-80 $\mu mol \cdot cm^{-2} \cdot s^{-1}$16 h. However, since August 2000, when the explants number of each accession and the number of accession increased, the collections were divided into two different groups cultivated either at 20 °C or at 25 °C with maximum number of 18 explants per *in vitro* clone. This division was based mainly on the environmental condition from which each accession was originated. Two terms of multiplication rate were calculated, namely theoretical and practical multiplication rates. Theoretical multiplication rate (TMR) defined as number of total explants possibly obtained divided by number of survived explants while practical multiplication rate (PMR) defined as number of total explants possibly obtained divided by the original number of explants at the beginning of the subculture. An explant was defined as a single node including one leaf taken from any part of the plantlet (excluding the original node). Three consecutive measurements were taken from October 2000 to March 2001 for the 25 °C group and from December 2000 to May 2001 for the 20 °C group, and the data were subjected to statistical analysis comparing the means of two independent samples using t-Test (Snedecor and Cochran, 1980).

Results

In Vitro *Introduction*

Germinated explants were on various stages of development after four weeks of cultivation. The latest stage of development for the two species are given in the Fig. 1 (taken 5 weeks after culture). ANOVA was conducted only for the formation of shoots and roots (germinated explants) since it is considered to be the most important developmental character for the *in vitro* introduction. The statistical analysis indicated that there was no interaction between genotype and 'introduction medium' used. The main effects of each treatment, therefore, are discussed.

***Genotype effect**:* The fact that Yam 16 was able to produce shoots and roots in this experiment (Table 1) is encouraging, since in our previous work of *in vitro* introduction no shoots and roots were obtained. In these studies (unpublished), bigger tubers (about 5 cm in diameter) of Yam 16 and Yam 22 (also *D. bulbifera* L.) were cut into sets of about 2x1.5 cm skin area and 1 cm thickness. Only callus (87.2% and 40.5% for Yam 16 and Yam 22, respectively) was produced and many explants were contaminated. In the present experiment, Yam 21 germinated faster and produced significantly higher shoot and roots compared to Yam 16 (Table 1). Special morphological characteristics of the tubers seemed to be one of the factors responsible for these differences. *D. bulbifera* has a trait of a smooth tuber surface while the tuber of *D. oppositifolia* has many papilla on its surface from which germination can take place, although, in most cases, germination occurs on the head of the tuber. In the previous *in vitro* introduction with Yam 25 (*D. sansibarensis* Pax) which also possesses many papilla on the tuber surface, we were able to obtain 28% germination (shoots and roots) (unpublished). Genotype influences on *in vitro* introduction have also been indicated by Malaurie *et al*. (1993) on their *in vitro* introduction using seeds and nodal cuttings.

Table 1: Effects of genotype and culture medium on the *in vitro* development of yams 4 weeks after start of the subculture[$]

Treatment	Survived explants	Shoots/ roots (%)[§]	Callus (%)	No reaction (%)	Contaminated (%)
Genotype					
Yam 16	85	16.77a*	8.2	68.2	21.3
Yam 21	94	68.52b	3.3	15.9	13.0
Medium					
I1	58	50.76a**	5.2	36	39.0
I2	54	42.16a	3.7	39	50.0
I3	67	35.01a	2.9	46	14.0

[$]) Contaminated explants were not included in calculating percentages of different developmental characters. [§]) Parameter analysed statistically . *) Numbers within the column below genotype followed by similar letter are not significantly different at the 5% level based on F-Test. **) Numbers within the column below medium followed by similar letter are not significantly different at the 5% level based on the F-Test.

Contamination level was generally lower compared to that of underground tubers of *D. trifida, D. rotundata and D. cayenensis* reported by Mitchell *et al*. (1995) who obtained an average contamination of 62% (with skin) and 32% (without skin), even though the explants were double sterilized. Contamination of the explants was higher (31.9%) on Yam 16 compared to that of Yam 21 (15%) (Table 1). Cutting of the tuber might have increased the vulnerability of Yam 16 to contamination. The same reason also explains the higher callus formation on Yam 16, since callus formed in this accession was wound callus, produced on the border of the cutting surface, while callus formation on Yam 21 occurs on papilla on the tuber surface (Fig. 2). Higher percentages of Yam 16 explants did not show any response (68.2%) compared to that of Yam 21 (15.9%) after 4 weeks of culture.

Medium effect: Although medium I1 showed slightly better effect (50.8%) on the production of shoots and roots compared to I2 (42.2%) and I3 (35.0%), this effect was statistically not significant (Table 1).

Figure 1: Latest morphological developmental stages of Yam 16 (*D. bulbifera* L and Yam 21 (*D. oppositifolia* L.) after 5 weeks of *in vitro* introduction using aerial tubers.

Increasing concentration of kinetin from 5 mg/l (I1) to 10 mg/l (M2) on MS salt medium did not increase the germination percentage of *in vitro* introduced aerial tubers. This finding was in agreement with that reported by Zok *et al*. (1998) who obtained high numbers of shoots with the kinetin concentration ranging from 5 to 10 mg/l on the *in vitro* introduction of *D. alata* L., *D. esculenta* (Lour.) Burk and *D. rotundata* Poir using nodal explants. Furthermore, supplementing MS medium (plus vitamins) with 0.2 mg/l NAA and 0.5 mg/l BAP (M3) instead of supplementing pure MS salt medium with IAA and kinetin (in I1 and I2) did not increase the percentage of aerial tubers' germination. I3 medium (with BAP and NAA) was used by Nair and Chandrababu (1994) to introduce *D. alata* L., *D. esculenta* (Lour.) Burk and *D. rotundata* Poir using nodal segments. Malaurie *et al*. (1993) used 1 mg/l NAA and 0.2 mg/l BAP to replace activated charcoal and glutamin in their maintenance medium (basal culture medium containing Knop's modified mineral nutrients, MS modified vitamins, 3% sucrose and 0.8% agar, 0.2% activated charcoal and 200 mg/l glutamin) as special 'introduction medium' for clones which were difficult to be introduced using maintenance medium, while Mitchell *et al*. (1995a) obtained high survival rates of meristem tips (83%) for *D. trifida* 'Yampie' using MS salts supplemented with 0.2 mg/l BAP and 1.0 mg/l NAA. Medium I2 (MS salts with 10 mg/l kinetin) exhibited the highest percentage of contamination (50%) followed by I1 (39.0%) and I3 (14.0%). Callus formation was generally low, although there was a small difference among the three media used. The percentage of non-responsive explants was slightly higher in medium I3 than in the other two media (Table 1).

Multiplication rates

It has been indicated that grouping the accessions in two different cultivation temperatures was based mainly on their geographical origin. Those cultivated in temperature of 20 °C are originated and distributed in sub-tropical and temperate areas such as North America (At-

lantic Yam - *D. villosa* L.) and South Eastern Europe (*D. balcanica* Kosanin; *D. caucasica* Lipsky) and used as medicinal plants. These species posses morphological characteristics of smaller stem diameter and smaller leaf sizes compared to those cultivated in 25 °C which originated and distributed in tropical areas of the Asian continent (Chinese Yam - *D. oppositifolia,* Greater Yam - *D. alata,* Potato Yam - *D. bulbifera*), the African continent (*D. sansibarensis, D. bulbifera, D. alata, D. cayenensis-rotundata*) and Central and Northern South America (Cush-cush Yam - *D. trifida,* Guinea Yam - *D. cayenensis-rotundata*), and cultivated mainly as food crops, although some of them are toxic and some others are used for medicinal purposes (Keller, 2001). An exception in our cultivation was that Yam 2 (*D. nipponica* Makino) which is distributed in Japan, Korea, northern and central China, former USSR and possesses similar morphological characteristics as those cultivated in 20°C, was cultivated in 25°C. This accession was used as a comparison to the tropical species. Almost all of the accessions of sub-tropical areas were introduced into *in vitro* culture with more than one clone, and four of these clones were lost in course of the maintenance. For some accessions, two clones were used in calculating multiplication rates. The second introduction of these accessions was considered as replication (Table 2).

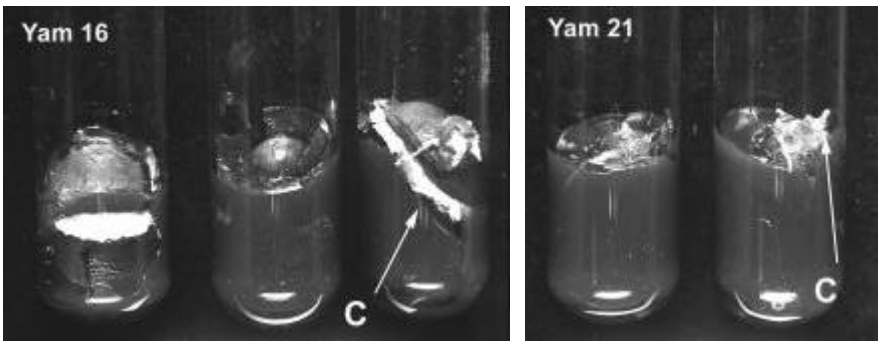

Figure 2: Callus (C) formation on Yam 16 (*D. bulbifera* L.) and Yam 21 (*D. oppositifolia* L.) explants from aerial tubers**.**

Accessions of the 20°C group (temperate and sub-tropical yams): Theoretically yams accessions, cultivated at 20°C, were able to multiply 2.8 ±1.48 to 5.61± 2.73 times (Table 2.). Yam 14 (*D. villosa*) has the highest multiplication rate and was statistically different compared to other accessions except to Yam 07 (*D. caucasica*). Other accessions were statistically not significantly different among each other. In practice, however, we were only able to obtain rates of 0.87±0.36 to 2.35±0.70. The lowest PMR was exhibited by Yam 10 (*D. caucasica*) which was significantly different compared to the other accessions. The highest PMR was shown by Yam 09 (*D. caucasica*), but this was not significantly different from that of Yam 04 (*D. villosa*), Yam 06 (*D. caucasica*), Yam 07 (*D. caucasica*), and Yam 14 (*D. villosa*) (Table 2). High percentages of not survived explants exhibited by Yam 10 seemed to be responsible for the lower *in vitro* PMR compared to accessions of similar species and even different species (Table 2). In six of nine accessions of temperate and sub-tropical yams, TMR was significantly higher than PMR. Only in three accessions, all of *D. caucasica,* TMR was similar to PMR, which was mainly due to lower percentages of not survived and/or contaminated explants exhibited by these three accessions (Table 2). Percentages of not survived explants of accessions cultivated in this temperature regime were generally high (16.7% -56.6%) which indicated that not all explants from the temperate and sub-tropical yams can be used as material for propagation.

Accessions of the 25 °C group (tropical yams): TMR of yams grown at 25 °C ranged from 3.05±1.34 to 6.26±3.76 (Table 2). The lowest value exhibited by Yam 15 (*D. oppositifolia*) and the highest by Yam 36 (*D. alata* 'Soglan'). Statistically, however, there was no signifi-

cant difference among all accessions. TMR shown by D. *cayenensis* (Yam 45) in our experiment was higher (4.78±2.92) than the multiplication rate of nodal explants of the cultivar RLYY of the same species grown in various establishment media, even after 12 weeks of sub culture (maximum of 4.3 for basal medium with 0.5 mg/l BAP and 0.05 mg/l NAA), reported by Mitchell *et al.* (1995b). For *D. trifida* 'Short Neck Yampie', however, they were

Table 2: Multiplication rate of various yams (*Dioscorea* spp.) cultivated in two different temperature regimes about 2 months after transfer

T	Acc.	Scientific name §	Donor	R	% NS	% C	TMR	PMR
20°C	Yam 01	*D. balcanica* Kosanin	BG Innsbruck	6	47.8	3.3	3.51±1.45a*/a**	1.69±0.75b*/b**
	Yam 04	*D. villosa* L.	BG Düsseldorf	6	36.1	1.03	3.02±0.92a/a	1.90±0.71bc/b
	Yam 06	*D. caucasica* Lipsky	BG Kiel	3	31.5	5.6	2.80±1.48a/a	1.69±1.02bc/a
	Yam 07	*D. caucasica* Lipsky	BG Frankfurt	3	37.1	6.2	4.33±2.62ab/a	2.33±0.13c/a
	Yam 08	*D. villosa* L.	BG Frankfurt	6	39.8	2.8	3.52±1.47a/a	1.70±0.39b/b
	Yam 09	*D. caucasica* Lipsky	BG Jena	6	16.7	0.9	2.86±0.88a/a	2.35±0.70c/a
	Yam 10	*D. caucasica* Lipsky	BG Poznan	6	65.3	4.0	3.43±1.98a/a	0.87±0.36a/b
	Yam 13	*D. balcanica* Kosanin	BG Bratislava	6	38.9	8.3	3.28±1.39a/a	1.45±0.30b/b
	Yam 14	*D. villosa* L.	BG Bratislava	6	56.6	1.0	5.61±2.73b/a	2.13±1.40bc/b
25°C	Yam 02	*D. nipponica* Makino	BG Innsbruck	3	77.6	0	3.62±3.31*/a**	1.12±0.69*/a**
	Yam 15	*D. oppositifolia* L.	BG Siena	3	1.9	4.4	3.05±1.34a/a	2.88±1.31abc/a
	Yam 16	*D. bulbifera* L.	BD Frankfurt	3	16.7	2.2	5.65±2.94a/a	4.72±3.12abc/a
	Yam 17	*D. sansibarensis* Pax	BG Frankfurt	3	1.9	0	3.35±1.45a/a	3.26±1.29bc/a
	Yam 18	*D. oppositifolia* L.	Coll. Sariwan Korea	3	2.2	0	3.32±0.36a/a	3.25±0.22bc/a
	Yam 19	*D. oppositifolia* L.	BG Ferrara	3	14.8	0	3.11±0.78a/a	2.65±0.71bc/a
	Yam 20	*D. japonica* Thunb.	BG Frankfurt	3	2.0	0	3.13±0.53a/a	3.07±0.54bc/a
	Yam 21	*D. oppositifolia* L.	BG Padua	3	0	0	3.76±0.65a/a	3.76±0.65bc/a
	Yam 22	*D. bulbifera* L.	BG Essen,	3	1.9	0	3.64±0.70a/a	3.57±0.74bc/a
	Yam 23	*D. sansibarensis* Pax	BG Berlin,	3	1.9	4.4	3.74±0.57a/a	3.54±0.74bc/a
	Yam 24	*D. bulbifera* L.	BG Mainz,	3	12.0	0	5.79±2.23a/a	5.10±2.03bc/a
	Yam 25	*D. sansibaresis* Pax	BG Cluj-Napoca	3	3.7	0	3.73±0.51a/a	3.57±0.20bc/a
	Yam 26	*D. oppositifolia* L.	BG Ljubljana	3	0	0	3.33±0.34a/a	3.33±0.34bc/a
	Yam 31	*D. sp.*	Mali	3	0	35.6	3.27±0.83a/a	2.20±1.06ab/a
	Yam 33	*D. sp.*	Costa Rica	3	2.2	0	3.70±1.33a/a	3.65±1.40bc/a
	Yam 34	*D. alata* L. 'Florido'	Ivory Cost	3	0	4.4	5.03±1.91a/a	4.77±1.42c/a
	Yam 35	*D. alata* L. 'Nza'	Ivory Cost	3	3.6	0	3.71±1.59a/a	3.64±1.60bc/a
	Yam 36	*D. alata* L. 'Soglan'	Ivory Cost	3	0	0	6.26±3.67a/a	6.26±3.67abc/a
	Yam 38	*D. alata* L. 'Suidie'	Ivory Cost	3	0	6.7	4.33±1.69a/a	4.08±1.57bc/a
	Yam 45	*D. cayenensis* Lam.	Ivory Cost	3	2.1	0	4.78±2.92a/a	4.62±2.79abc/a
	Yam 48	*D. trifida* L. 'Yam Pie'	Jamaika	3	2.0	4.4	3.68±1.91a/a	3.51±1.74abc/a
	Yam 53	*D. sp.*	Magdeburg (market)	3	20	0	3.36±0.71a/a	2.90±1.09abc/a

§) Some names have been either changed from or confirmed as the original name obtained from the donor sources after identification, *) Numbers (values± standard errors) within the column (between accessions) followed by same letter (s) are not significantly different at the 5% level based on t-Test. **) Numbers (values± standard errors) within the row (within accessions) followed by same letter (s) are not significantly different at the 5% level based on t-Test. BG = Botanic Garden, C = contaminated explants, NS = Not survived explants, PMR = Practical multiplication rate, R = Number of replicates, T = Temperature, TMR = Theoretical multiplication rate.

able to obtain a multiplication rate of 5.0 after 4 weeks of subculture on basal medium supplemented with 0.5 mg/l BAP using nodal explants initiated from small tubers which was higher than our finding on *D. trifida* 'Yampie' of 3.68±1.91 (Table 2). These differences may be attributable to different media used and different methods of determining the MR (TMR). Mitchell *et al.* (1995b) defined MR as summation of original node, new nodes and

shoots, while we used only new nodes and shoots. Practically, only factors of 1.12±0.69 to 6.26±3.67 times can be produced by accessions grown in this temperature. However, the lowest value belongs to Yam 02 (*D. nipponica* Makino) which is actually an accession of the sub-tropical area and showed high percentages of not survived explants (77.6%). This accession was significantly weaker than most of the accessions cultivated in the same temperature. The other accessions showed similar PMR except between Yam 34 (*D. alata* 'Soglan') and Yam 31 (*D.* sp.) which is mainly due to the high contamination percentage (35.6%) exhibited by Yam 31 (Table 2). All accessions cultured at 25 °C showed similar TMR and PMR indicating that tropical yams can be propagated *in vitro* using nodal explants from any part of the plant. Mitchell *et al.* (1995b) used explants of various origin in *D. cayenensis* and *D. trifida.* They found that older nodes from plantlets grown *in vitro*, regardless of their origin, produced more shoots. Younger nodes grew weaker, but there was more swelling and bud development on nodal regions which, in the next subculture, resulted in higher growth.

Discussion

Traditionally, genetic conservation of vegetatively propagated crops including *Dioscorea* spp. has been done mainly through field cultivation. Field maintenance, however, cannot guarantee the security of the collection, since the loss can occur through pests and diseases, virus, poor sprouting, unfavourable storage conditions, drought and poor handling, which has been reported for yams to achieve 10% annually (Okoli, 1991). Beside this, it is also time and space consuming and laborious. *In vitro* genebanks, therefore, offer a better way of conserving genetic diversity of such crops. To be able to conserve yams through *in vitro* culture using slow growth conditions and cryopreservation, as well as for other purposes such as rapid multiplication, producing virus free plantlets through meristem culture, and germplasm exchange, *in vitro* introduction would be the first step. This has to be followed by the establishment of the cultures for which information on the multiplication rates of the introduced clones is important. Previous research has shown that *in vitro* sowing gave the highest rate of success followed by introduction of nodal cuttings (Malaurie *et al*., 1993). But yam (*Dioscorea* spp.) does not always produce seeds in the field (Ng and Daniel, 2000). Meristem tips have also been used with high success as primary explants. It takes, however, slightly longer periods (28 weeks) to produce plantlets (Mitchell *et al*., 1995a). Attempts to introduce yams using underground tubers have not been successfully done, since all the cultures became necrotic within eight weeks of culture (Mitchell *et al*., 1995a). Our experiment indicated, that yam can be introduced into *in vitro* culture by means of aerial tubers in a quite short time and that cultivating whole tubers of small size would be more suitable than sets of cut tubers, which results in high contamination and callus production. For yams which produce aerial tubers in the field, therefore, this organ can be used as a material for *in vitro* introduction. The experiment also evaluated three different culture media which have been used for introduction and establishment (with slight modification). In I1 and I2 different concentrations of kinetin combined with NAA were used to supplement MS basal medium (Zok *et al*., 1998), while in I3, BAP and IAA were used to supplement MS medium (Nair and Chandrababu, 1994). The results indicated that there was no statistical difference between these three media, although I1 medium gave slightly greater effect, followed by I2 and I3, respectively. This finding was in contrast with that of Mitchell *et al.* (1995a), who reported that *D. cayenensis, D. trifida* and *D. rotundata* are more responsive to BAP than to kinetin for shoot production. This may, however, be explained by lower concentrations of BAP and NAA used in our case. Furthermore, the species and materials used in these studies were different. Information on multiplication rates is not only important for commercial purposes of rapid multiplication. It is also useful to support decisions of whether certain measurements should be taken in case of a given accession for its safe germplasm preservation.

Theoretical multiplication rate represent genetic potential of the accession to reproduce *in vitro* in the next subculture. Practical multiplication rate, on the other hand, shows environmental influences on the genetic potential which in this case including selection of the explants, culture media used, ability of the accessions to withstand diseases, and the technical work of sub-culturing. Several studies have been conducted on multiplication rates of yams (Mitchell *et al*., 1995 a, b; Zok *et al*., 1998). Those studies, however, were focused only on some edible, tropical yams and on the theoretical multiplication rate. Our study indicated that yams originated and distributed in sub-tropical and temperate areas are able to produce almost as many explants as tropical yams. However, not all of these explants can reproduce in the next subculture on the culture medium used for this study. For these accessions, therefore, care should be taken over the selection of explants, and further research should be done aiming at improving medium and other conditions for their multiplication. For tropical yams, on the other hand, it seems that all node explants regardless of their position are able to reproduce upon subculture.

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